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Joint action of a juvenile hormone analogue and selected molt
disruptants on the western spruce budworm, Choristoneura
occidentalis Freeman

FINAL REPORT

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1. Summary

Diet incorporation experiments determined the lethal effectiveness of a juvenile hormone analogue, molt disruptants, and 1:9 mixtures of the juvenile hormone analogue with the molt disruptants to 4th and 6th instar western spruce budworm, Choristoneura occidentalis Freeman. No dissimilar joint action of the two types of insect growth regulators was observed in experiments with 4th instars; however, this type of joint action was observed with methoprene + Dimilin and methoprene + EL-7063 ingested by 6th instars. These two combinations merit further investigation as candidates for applications on populations consisting of mostly 6th instars.

2. Introduction

A. Justification: Methoprene, a juvenile hormone analogue, and molt disruptants (MDs) such as Dimilin and BAY SIR 8514 share a common deficiency in regard to their potential use for control of spruce budworms. Although environmentally safe, they are comparatively ineffective or only marginally effective on western or eastern spruce budworm. This investigation was necessary to provide basic toxicological data to evaluate the feasibility of combining the two types of insect growth regulators (IGRs) into a mixture with sufficient efficacy to be both economical and practical.

B. Objectives: This study addressed the following questions: (1) Can the activities of MDs such as Dimilin be enhanced by the presence of methoprene when last instar western spruce budworm (WSBW) are the primary spray target? (2) Can the activities of MDs be enhanced by methoprene when 4th instars are the primary target and exposure to the mixture lasts only for the duration of the 4th instar?

3. Methods

A. Insects: Newly molted 4th and 6th instar WSBW were selected at random from the 84th-86th generations of a nondiapausing laboratory colony (Robertson, 1979). Sufficient eastern spruce budworm were not available for experiments as originally planned.

B. Formulation: All formulations were prepared in distilled water on the basis of weight/volume concentrations of the active ingredient. Mixtures consisted of 1:9 proportions of methoprene: MD. The chemicals and formulations were:

<u>Chemical</u>	<u>Formulation</u>
methoprene	6 lb/gal emulsifiable concentrate (EC)
diflubenzuron (Dimilin)	TH 6040 25% wettable powder
BAY SIR 8514	25% wettable powder
EL-7063	50% wettable powder

C. Experimental Design: Each of the six bioassays was blocked by replication. The

First replication tested a wide series of logarithmic concentrations in order to bracket the effective concentration range. Within this range, 8 concentrations were chosen ^{FOR TESTING} in 3 subsequent replications. Each block of replications for each instar was completed before the next began. Within blocks, experiments were divided into sets. Each set consisted of methoprene alone, the MD alone, and a 1:9 mixture of methoprene + MD. Within a set, groups of 10 insects were randomly assigned to specific chemical-concentration combinations. Use of sets assured that results would not be confounded by possible intergenerational shifts in response (Savin et al., 1977). This precaution was particularly important because of the multiple generations spanned by these experiments.

D. Bioassay Procedures: The ingestive mode of effectiveness was tested for both 4th and 6th instars. Each chemical concentration was mixed into reliquified artificial diet as described by Robertson and Boelter (1979). The 10 insects in each treatment group were provided with a 10-ml quantity of treated diet and held within a 100 x 20-mm plastic petri dish lined with filter paper. Sixth instars were removed from the treatment dishes as pupae. Fourth instars were held for 5-7 days, until the fifth instar was attained; the insects were then transferred to fresh artificial diet for the completion of their larval development. All pupae from each treatment group were held in ~~AN INDIVIDUAL~~ 60 x 15-mm petri dish until death or adult emergence.

E. Data Analysis: Concentration-mortality lines for each treatment were computed with the probit option of POLO (Russell et al., 1977). The position of the methoprene + MD line relative to the three reference lines for independent joint action, described by Plackett and Hewlett (1948) and Hewlett and Plackett (1950), were determined. A statistical assessment was then made to estimate whether joint action had occurred and, if so, whether it was dissimilar joint action. Dissimilar joint action is the ideal type, and indicates that a mixture is far more active than

either of individual components.

4. Results

A. Diet Incorporation Tests - 6th instars: Dissimilar independent joint action, with negative correlation of the tolerances of methoprene and the MD, was observed with methoprene + Dimilin and, to a greater extent, with methoprene + EL-7063 (Table 1). With these combinations, the lethal effectiveness of the mixture was clearly greater than the lethal effectiveness of the most toxic of the individual components. The correlation of tolerance to methoprene and BAY SIR 8514 approximated $\rho = 0$; the effectiveness of the mixture was less than that of methoprene, the most toxic of the components.

B. Diet Incorporation Tests - 4th instars: No independent joint action of methoprene with any of the MDs was observed (Table 2). In all cases, the mixture was less effective than the MD, the most toxic of the two components.

C. Significance and Interpretation of Results: Independent joint action has been described statistically by Bliss (1938), Plackett and Hewlett (1948), and Hewlett and Plackett (1950). In this type of joint action, the most desirable situation occurs when the effects of the mixture components are negatively correlated. As ρ approaches -1 , insects affected by one of the chemicals are not affected by the other chemical and visa versa. The total effect is, therefore, greatly enhanced toxicity of both components. When values of ρ range from 0 to $+1$, less desirable joint action is described: both chemicals act on the same insects, and no enhancement in total effect occurs. Values of ρ which are negative estimate dissimilar joint action, while positive values estimate similar joint action.

Our experiments showed that dissimilar independent joint action, with negative values of ρ , occurred with mixtures of methoprene + Dimilin or EL-7063 ingested by last instars. A mixture of methoprene + BAY SIR 8514 demonstrated the less desirable, similar, joint action. Experiments with 4th instars indicated that none

Table 1. Toxicity of methoprene, MDs, and 1:9 mixtures of methoprene with MDs to 6th instar WSBW. Mortality recoded at adult eclosion.

Chemical	Number treated ^{a/}	Slope $\pm$ S.E.	LC ₅₀ (parts per million)
A. Methoprene, Dimilin			
Methoprene	477	0.88 $\pm$ 0.11	1.70
Dimilin	459	0.54 $\pm$ 0.09	5.41
M + D (1:9)	320	1.18 $\pm$ 0.14	3.92
B. Methoprene, BAY SIR 8514			
Methoprene	480	1.35 $\pm$ 0.13	1.04
BAY SIR 8514	480	1.41 $\pm$ 0.14	1.52
M + B (1:9)	320	1.94 $\pm$ 0.20	1.72
C. Methoprene, EL-7063			
Methoprene	480	1.17 $\pm$ 0.13	1.02
EL-7063	360	1.84 $\pm$ 0.25	0.30
M + E (1:9)	440	3.45 $\pm$ 0.91	0.15

^{a/} Mortality in concomitantly treated control groups of 40-60 insects ranged from 0.00 $\pm$ 0 to 0.072 $\pm$ 0.051.

Table 2. Toxicity of methoprene, MDs, and 1:9 mixture of methoprene with MDs to 4th instar WSBW. Mortality tallied at adult eclosion.

Chemical	Number treated ^{a/}	Slope $\pm$ S.E.	LC ₅₀ (parts per million)
A. Methoprene, Dimilin			
Methoprene	467	0.08 $\pm$ 0.21	not toxic
Dimilin	476	1.64 $\pm$ 0.61	6.35
M + D (1:9)	475	0.43 $\pm$ 0.16	13.00
B. Methoprene, BAY SIR 8514			
Methoprene	395	1.52 $\pm$ 0.53	161
BAY SIR 8514	430	0.28 $\pm$ 0.13	206
M + B (1:9)	381	0.21 $\pm$ 0.17	4500
C. Methoprene, EL-7063			
Methoprene	410	0.30 $\pm$ 0.19	11,000
EL-7063	210	1.35 $\pm$ 0.35	0.17
M + E (1:9)	400	2.50 $\pm$ 0.15	0.31

a/ Mortality in concomitantly treated control groups of 29-30 insects ranged from 0.036 $\pm$ 0.036 to 0.167 $\pm$ 0.068.

of the mixtures had any advantageous joint action.

5. Conclusions and Recommendations

EL-7063 and Dimilin, mixed with methoprene, should be tested further. Experiments to estimate and predict their LD_{90} s on foliage and simulated aerial application experiments should be performed. Future experiments with these mixtures should emphasize applications to 6th instars, since early application appeared impractical from our results. However, the possibility that sufficient residual activity on foliage could overcome this lack of effect on 4th instars should not be disregarded.

6. Suggestions for Additional Research

These experiments have demonstrated significant enhancement of MD activity by a juvenile hormone analogue. The physiological mechanism underlying this enhancement should be investigated so that rational choices of other mixture components can be made.

Additional laboratory experiments should be conducted to determine the effects of methoprene + Dimilin or methoprene + EL-7063 applied to foliage on which WSBW are foraging at the time of application. Precise estimation of dosages needed for 90% control should be done as the next logical step in the sequence leading to simulated aerial application experiments or hydraulic application trials in special use areas.

7. Manuscript Planned

A technical manuscript describing these experiments is in preparation for submission to J. Econ. Entomol.

8. Appendix: References Cited

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